Primary cilia dysfunction as a novel pathogenic mechanism of birth defects in FASD

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• Karen Boschen has no conflicts of interest to report.
A MOUSE MODEL OF FETAL ALCOHOL SPECTRUM DISORDERS (FASD)

MRI images from:
Godin et al., 2010 & Parnell et al., 2009

<table>
<thead>
<tr>
<th>Gestational Week in Humans</th>
<th>Gestational Day in Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
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Gastrulation

FAS

Control

PAE

Child's brain after Prenatal Exposure to Alcohol:
- Smaller head size
- Smaller brain
- Flattened nose
- Underdeveloped inner structure of brain

Gestational Week in Humans
- 2 weeks
  - 6 days
- 3 weeks
**How does alcohol affect face and brain development?**

**Sonic hedgehog pathway:**
- Protein critical for face and brain development, particularly along the ventral midline

**Cell death:**
- Gastrulation: Downward arrow
- Neurulation: No change

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**Xavier et al., 2016 & Liu et al., 2014**

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SONIC HEDGEHOG (SHH) PATHWAY IN THE ROSTROVENTRAL NEURAL TUBE (RVNT)

Does neurulation-stage alcohol exposure affect the Shh pathway in the RVNT?

C57BL/6J x C57BL/6J

PAE (2.9 g/kg) x 2
Vehicle

Gestational Day
9 9.25 9.5 10

Shh pathway
SHH PATHWAY IS DOWNREGULATED 6-12 HR AFTER PAE

Boschen et al., in preparation

SHH pathway

Gene Transcription

Log2 Fold Change vs. Age-matched Controls

Gli1
Gli2
Gli3
Smo

Shh

Shh pathway

GD9.25
GD9.5

Percent of Total Gli3

Gli3 - Repressor
Gli3 - Activator

Vehicle
NAE
Vehicle
NAE

Boschen et al., in preparation
REduced SHH-Mediated Cell Cycle Genes and RVNT Volume 6-12 HR After PAE

**Cell Cycle Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Log2 Fold Change vs. Age-matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccnd1</td>
<td>-1.5</td>
</tr>
<tr>
<td>Ccnd2</td>
<td>-1.0</td>
</tr>
<tr>
<td>Fgf15</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

**Cell Proliferation**

**Gene Transcription**

Gli1 → Gli2 → Gli3 → Nucleus → Ccnd1, Ccnd2, Fgf15 → Cell Proliferation

**RVNT Volume**

- GD9.25 vs. GD9.5
- **Cell layer width (µm)**
- **Volume (mm³)**

**Control** vs. **Alcohol**

- GD9.25
- GD9.5

Boschen et al., in preparation
SHH TRANSDUCTION REQUIRES PRIMARY CILIA

- Shh pathway requires functioning primary cilia: hair-like sensory organelles that protrude from most cells
- Important for developmental processes, e.g. organogenesis, limb development, neural patterning
Genetic ciliopathies affect primary cilia structure, function, or cilia-anchoring proteins.

Ciliopathies affect many organ systems, including development of the brain, orofacial region, and digits:
- Eye defects (e.g. coloboma)
- Cleft palates, lips
- Polydactyly

Associated with Shh pathway dysregulation.
ANIMAL MODELS OF CILIOPATHIES

- Target cilia-related proteins, particularly those involved in Shh signaling
- Eye defects, cleft palate and lips, polydactyly, hypo/hypertelorism

Chang et al., 2016
PRIMARY CILIA DENSITY WAS NOT AFFECTED BY PAE

What about primary cilia function?

Whole transcriptome analysis
PAE ALTERS CILIATION-RELATED GENE EXPRESSION IN THE RVNT

- Upregulated
- Downregulated

Genes related to:
- Ciliogenesis
- Cilia structure
- Protein trafficking
- Shh signaling
- Cell cycle
- Genetic ciliopathies
1. Dysregulation of Shh pathway in regions of the neural tube that give rise to ventral midline brain structures could disturb the growth trajectory of these areas, resulting in both physical malformations and perturbed cognitive-behavioral function.

2. Based on the changes in expression of Gli3 and genes related to cilia function and stability, we hypothesize that neurulation-stage alcohol induces a “transient” ciliopathy in the embryo, leading to the shared phenotype between ciliopathies and prenatal alcohol exposure.
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SHH AND FACIAL WIDTH

Brugmann 2010
Cilia retract during active mitosis
Mother centriole becomes cilia’s basal body
MOTILE CILIA IN THE PRIMITIVE NODE

Sulik et al., 1994